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A Novel Hard Clam Parasite: Making Sense of a New Finding

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ABSTRACT

During a routine histopathological examination of 180 juvenile hard clams, *Mercenaria mercenaria*, from a site in Virginia, USA in 2007, we discovered a single individual heavily infected with what appeared to be a haplosporidian parasite. The Haplosporidia include species causing lethal oyster diseases. SEM of spores indicated that the parasite belonged to the genus *Minchinia*. Sequencing of the SSU rRNA gene confirmed that it is a previously unknown *Minchinia* species closely related to *M. tapetis*, a parasite of the European clam, *Ruditapes decussatus*. Further sampling of clams near the area of the first discovery found prevalences up to 100% using PCR. No detectable parasites were found in routine screening of the same individuals using tissue-section histology with H&E staining. No unusual mortalities have occurred among the sampled groups. PCR analysis of juvenile clams from Florida in 2007, and Massachusetts, New Jersey and North Carolina in early 2008 failed to detect the parasite. On the other hand, an unidentified haplosporidian found in a hard clam from New Jersey in 2001 has since been identified as the new *Minchinia* sp. and another, from Maine in 1999, was infected with what may be the same parasite. These findings suggest that the parasite may be geographically widespread, but only very rarely develops infections detectable by routine histology. The discovery underscores critical questions about molecular assays that signal the presence of a parasite, but not necessarily infections detectable by more traditional diagnostic assays.

INTRODUCTION

The northern quahog or hard clam, *Mercenaria mercenaria*, is among the most valuable North American commercial shellfish species. Along the eastern coast of the United States, *M. mercenaria* production was worth approximately 50 million US dollars in 2006 (http://www.st.nmfs.noaa.gov/st1/commercial/landings/annual_landings.html). Much of the production is from aquaculture. The success of this industry is due partly to its relative freedom from disease. The only serious disease agent of hard clams is the parasite QPX (Quahog Parasite Unknown), which has damaged *M. mercenaria* production in New England (Smolowitz et al., 1998). Production from the midAtlantic and Southeastern United States has experienced little or no negative impact (Ragone Calvo et al., 1998). Most states now require health inspections of clams before they can be shipped from a hatchery or nursery in one state to a grower in another state. The diagnostic assays used are typically Ray's Fluid Thioglycollate Medium culture for *Perkinsus* spp. and tissue section histology for other parasites and pathological conditions. In fall 2007, the Haskin Shellfish Research Laboratory (HSRL) at Rutgers University, New Jersey, received a shipment of 180 seed clams, produced that spring and grown in a Virginia nursery. Histological examination revealed one individual with a heavy infection of what appeared to be a haplosporidian parasite. Because the Haplosporidia include several species that are highly lethal to oysters, it was important to identify the organism, to determine its geographic distribution, and to assess its potential effect on the hard clam industry.

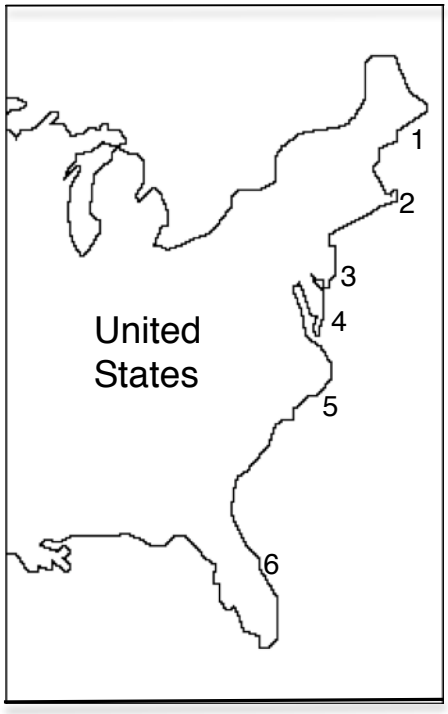

Figure 1. Locations sampled: 1 – Maine; 2 – Massachusetts; 3 – New Jersey; 4 – Virginia; 5 – North Carolina; 6 - Florida

MATERIALS AND METHODS

Molecular identification

Sections of the clam with the unknown parasite were sent to the Molluscan Disease Laboratory at the Virginia Institute of Marine Science (VIMS), which is the OIE (Office International des Epizooties) reference laboratory for Haplosporidia. There they were assayed by *in situ* hybridization with a generic haplosporidian probe. DNA isolated from the section was amplified with generic haplosporidian primers and the resulting PCR product sequenced. Specific primers for PCR amplification and probes for *in situ* hybridization were designed based on the sequence analysis.

Scanning electron microscopy

Embedded material containing spore stages was recovered from a paraffin block, rehydraded, and sonicated to disrupt tissues and release the spores. The spore suspension was then dehydrated, critical point dried, mounted on stubs, coated with gold-palladium, and observed with a scanning electron microscope.

Temporal and spatial distribution

Additional samples of the same group of clams were obtained from the Virginia site in October 2007 and in March 2008. Samples of seed clams were also obtained from Florida in October 2007 and from Massachusetts, New Jersey, and North Carolina in February, 2008. Individual clams were sectioned and fixed both in EtOH for PCR amplification and in Davidson's fixative for histology. In total, 1307 clams from 7 sites in 5 states (Fig. 1) were examined by one or more methods.

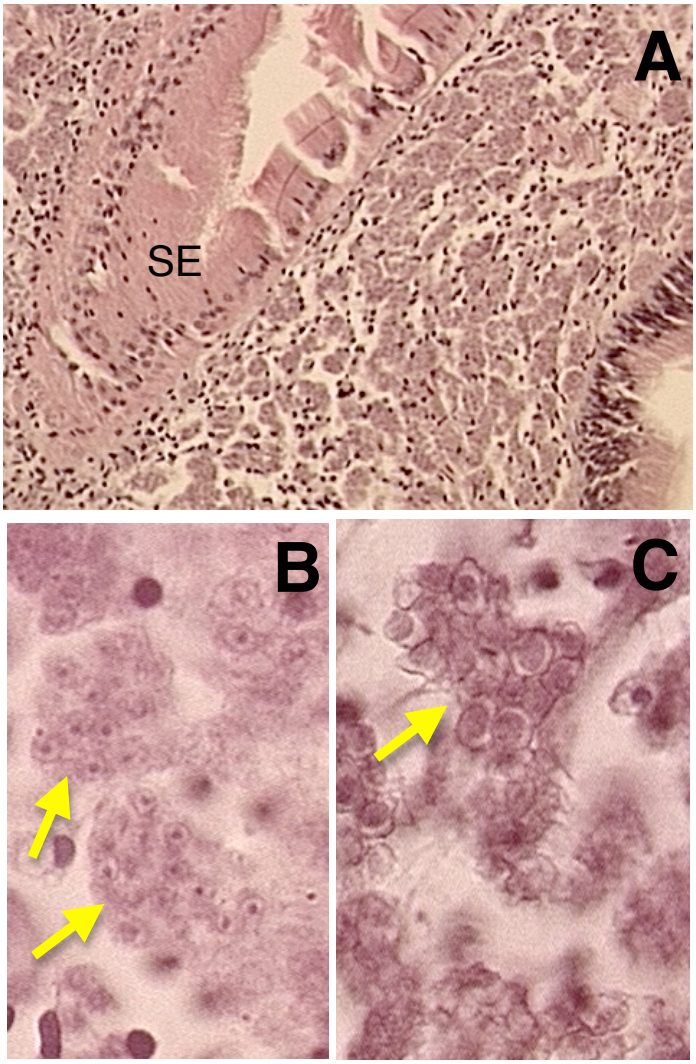

Figure 2. (A) Section showing massive concentration of *Minchinia* sp. plasmodia in the connective tissue of *Mercenaria mercenaria*. Small dark objects are hemocyte nuclei; larger, lighter staining objects are plasmodia. SE – stomach epithelium. (B) Two plasmodia (arrows) with nuclei showing central nucleolus. (C) Sporocyst with developing spores.

RESULTS

Light microscopy of the original infected clam revealed a massive infection in all tissues except the epithelium. Plasmodial, prespore, and spore stages were present (Fig. 2). Scanning electron microscopy of the spores confirmed the parasite to be a haplosporidian. At one end, an opening was capped by a hinged operculum surrounded by 2 or 3 short appendages (Fig. 3). A single, longer, tail projected from the aboral end. The appendages originated from the epispore cytoplasm. These general characteristics placed the organism in the genus *Minchinia* (McGovern and Burreson, 1990), but they were also unique enough to signal a new species.

Results of molecular assays, including sequencing of the SSU rRNA gene, also placed the parasite in the genus *Minchinia* and were consistent with the SEM results in designating it as a new species. Sequence comparison with other *Minchinia* spp. indicated that it is most closely related to *M. tapetis*, a parasite of the European clam, *Ruditapes decussatus*, in western Europe (Azevedo, 2001). Three other *Minchinia* spp. have been described. They parasitize ship worms, chitons, and scaphopods (Ball, 1980; Desportes and Nashed, 1983; Hillman et al., 1990).

When the specific molecular assays were employed, the prevalence of the new *Minchinia* sp. increased markedly in the cohort in which the original, histologically detected infection was found. *In situ* hybridization found parasite DNA in 10% of the sample, and PCR, in 37%. Another sample of seed clams from the vicinity of the first sample was 100% positive by PCR. On the other hand, samples from Massachusetts, New Jersey, North Carolina and Florida (N = 380) and two additional sites in Virginia (N=230) were negative by PCR.

DISCUSSION

In the present series of collections, the newly described *Minchinia* sp. was found only in clams that had been field-deployed at one site on the seaside of Virginia. Sampling at other Virginia sites as well as sites in Massachusetts, New Jersey, North Carolina and Florida, although admittedly limited, failed to detect evidence of the parasite in PCR assays. Histological examinations of hard clam seed for interstate transport have been conducted in the US at least since 1995 (Ford et al., 1997). Prior to the recent findings, only two individuals among many thousands of seed clams examined by several laboratories, was found with a parasite that resembled the new *Minchinia* sp. One was found in 1999 and came from the state of Maine. A second, adult, clam collected in 2001 during a New Jersey field trial, but from a cohort that had been produced and nurseried in Virginia for several months, had a haplosporidian that reacted positively when assayed *in situ* with the *Minchinia* sp. probe.

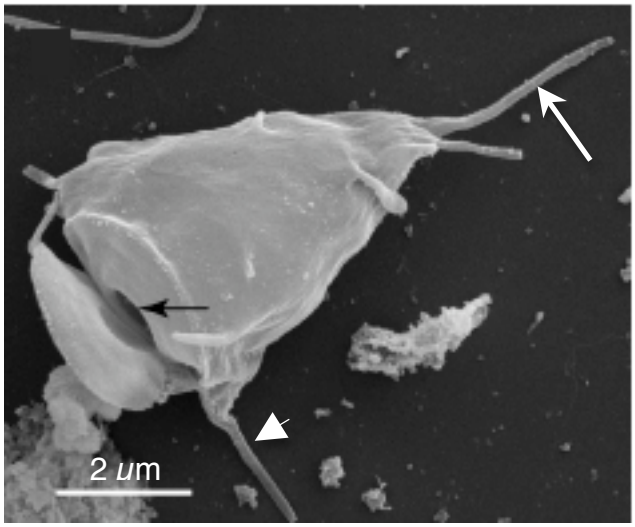

Figure 3. Scanning electron microscope image of *Minchinia* sp spore showing orifice covered by operculum (black arrow), aboral "tail" (white arrow, and oral appendage (white arrowhead).

We are unaware of any disease outbreaks or atypical mortalities in hard clams associated with any parasite except QPX, even though what is the same *Minchinia* sp. has now been found in clams from Virginia and New Jersey, and possibly Maine. The seed clams from Virginia with high PCR-positive prevalences have not experienced unusual deaths. Thus, we have no evidence that the parasite is a problem for hard clams at the population level, although infections can clearly become heavy in some individuals. Of more concern is the high prevalence found by PCR in some samples and the fact that of the 132 individuals that were positive by PCR and also examined by tissue-section histology, only one had an infection detected by routine histology.

Our findings illustrate what is likely to become an increasingly burdensome problem for shellfish culturists who ship molluscan seed or broodstock between states (and countries) and for the regulators who must make decisions based on diagnostic assays. How do they interpret PCR positive results without visual confirmation of infections? Or, if the parasite is seen by histology, but the PCR prevalence far exceeds the histological prevalence, as we report here,

what level of concern is appropriate and what regulatory action should be taken? Does the higher PCR prevalence represent dead parasites or parasites that are present on the surface of tissues or passing through the gut, but not in established infections? What is a realistic assessment of such findings that balances the need to minimize harm with the desire to support an industry?

It seems reasonable that efforts must be made to better evaluate the significance of such findings; however, we have found that granting agencies are reluctant to fund projects on issues that "are not (currently) causing a problem". Many in the industry feel that "studying" a topic like this will only precipitate a problem that doesn't exist. One can sympathize with their point of view, given the precautionary principle that many regulators follow. The principle prevents importation if any evidence of a pathogen or disease is found. Yet this "interpretation issue" is not going to disappear and sooner or later will have to be addressed in a scientifically rigorous manner that is understandable by both regulators and the industry.

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